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PGC-1α Serine 570 Phosphorylation and GCN5-mediated Acetylation by Angiotensin II Drive Catalase Down-regulation and Vascular Hypertrophy*[S]

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Shiqin Xiong[‡], Gloria Salazar[‡], Alejandra San Martin[‡], Mushtaq Ahmad[‡1], Nikolay Patrushev[‡], Lula Hilenski[‡], Rafal Robert Nazarewicz[‡], Minhui Ma[‡], Masuko Ushio-Fukai[§], and R. Wayne Alexander[‡¶2]

From the [‡]Division of Cardiology, Department of Medicine, and [¶]Emory University Hospital, Emory University School of Medicine, Atlanta, Georgia 30322 and the [§]Department of Pharmacology, University of Illinois at Chicago, Chicago, Illinois 60612

Angiotensin II (Ang II) is a pleuripotential hormone that is important in the pathophysiology of multiple conditions including aging, cardiovascular and renal diseases, and insulin resistance. Reactive oxygen species (ROS) are important mediators of Ang II-induced signaling generally and have a well defined role in vascular hypertrophy, which is inhibited by overexpression of catalase, inferring a specific role of H_2O_2. The molecular mechanisms are understood incompletely. The transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is a key regulator of energy metabolism and ROS-scavenging enzymes including catalase. We show that Ang II stimulates Akt-dependent PGC-1α serine 570 phosphorylation, which is required for the binding of the histone acetyltransferase GCN5 (general control nonderepressible 5) to PGC-1α and for its lysine acetylation. These sequential post-translational modifications suppress PGC-1α activity and prevent its binding to the catalase promoter through the forkhead box O1 transcription factor, thus decreasing catalase expression. We demonstrate that overexpression of the phosphorylation-defective mutant PGC-1α (S570A) prevents Ang II-induced increases in H_2O_2 levels and hypertrophy ([^{3}H]leucine incorporation). Knockdown of PGC-1α by small interfering RNA promotes basal and Ang II-stimulated ROS and hypertrophy, which is reversed by polyethylene glycol-conjugated catalase. Thus, endogenous PGC-1α is a negative regulator of vascular hypertrophy by up-regulating catalase expression and thus reducing ROS levels. We provide novel mechanistic insights by which Ang II may mediate its ROS-dependent pathophysiologic effects on multiple cardiometabolic diseases.

Reactive oxygen species (ROS)[3] are important mediators of cell senescence, neurodegenerative disease, cancer, cardiovascular diseases, and the metabolic syndrome (1–3). ROS are generated from multiple sources including mitochondrial metabolism and NADPH oxidases in response to extracellular stimuli including growth factors and hormones (4). Ang II is a pleuripotential hormone and a potent mediator of arterial hypertrophy, a hallmark of vascular wall remodeling in hypertension and of metabolic diseases such as type II diabetes and atherosclerosis (5, 6). These effects are mediated, in large part, through the G protein-coupled AT_1 receptor (7). Many growth-related outputs of the AT_1 receptor including hypertrophy are dependent upon the production of ROS, particularly H_2O_2, because overexpression of catalase, a scavenger of H_2O_2, inhibits Ang II-induced vascular hypertrophy *in vitro* and *in vivo* (8–10). Short term upstream signaling pathways that mediate Ang II-induced production of H_2O_2 have been well described (11). Ang II also stimulates sustained increases in ROS levels for 48–72 h that are associated with vascular smooth muscle cell (VSMC) hypertrophy (12, 13). ROS levels could be increased either (or both) by promoting generating capacity or/and by decreasing levels of scavenging enzymes, such as catalase. In cardiomyocytes, Ang II- and insulin-stimulated hypertrophy is ROS-dependent and is associated with down-regulation of catalase expression (14, 15). In mesangial cells, ROS stress reduces catalase transcription via the FoxO1 transcription factor (16).

Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is a transcriptional coactivator that was initially identified as a peroxisome proliferator-activated receptor γ-interacting protein from brown fat (17). Gene deletion studies in mice demonstrated that PGC-1α is a central regulator of ROS metabolism (18), energy homeostasis (19–22), heart failure (23–25), and postnatal angiogenesis (26). PGC-1α protects from oxidative stress by increasing expression of various antioxidant defense enzymes including catalase, copper/zinc superoxide dismutase, manganese superoxide dismutase, and glutathione peroxidase (18, 27). PGC-1α interacts with forkhead transcription factor 1 (FoxO1) and coactivates FoxO1-dependent gene expression (28–30). FoxO transcription factors are downstream targets of Akt, and their overexpression pro-

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[1] Present address: Cardiovascular Research Institute, Morehouse School of Medicine, 720 Westview Dr., Atlanta, GA 30310.

[2] To whom correspondence should be addressed: Division of Cardiology, Emory University Hospital, Suite H-153, 1364 Clifton Rd. NE, Atlanta, GA 30322. Tel.: 404-727-1749; Fax: 404-727-3099; E-mail: ralexan@emory.edu.

[3] The abbreviations used are: ROS, reactive oxygen species; Ang II, angiotensin II; VSMC, vascular smooth muscle cells; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; FoxO, forkhead box O; DN, dominant negative; DBE, DNA-binding element; ChIP, chromatin immunoprecipitation; siRNA, small interfering RNA; PEG, polyethylene glycol-conjugated; DCFDA, dichlorofluorescin diacetate; DMEM, Dulbecco's modified Eagle's medium; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT, reverse transcription; ERK, extracellular signal-regulated kinase; wt, wild type.

tects against oxidative stress (31) and inhibits cardiac hypertrophy (32, 33) at least in part by transcriptionally activating catalase (15). Thus, Ang II-induced hypertrophy is associated with inhibition of catalase transcription in VSMC. There is incomplete understanding of the mechanisms involved.

Post-translational modifications regulate the function and activity of PGC-1α at multiple levels. For example, transcriptional regulation of gluconeogenesis and fatty acid oxidation are suppressed by PGC-1α Ser^{570} phosphorylation by Akt, thus inhibiting PGC-1α recruitment to its cognate promoters (34). Conversely, AMP-activated protein kinase-dependent PGC-1α phosphorylation at Thr^{177} and Ser^{538} promotes transcriptional activity for genes regulating mitochondrial biogenesis, GLUT4, and PGC-1α itself (35). Further, lysine acetylation of PGC-1α by the histone acetyltransferase GCN5 (general control nonderepressible 5) decreases PGC-1α activity to control glucose metabolism (36). Moreover, PGC-1α deacetylation by SIRT1 (silent mating type information regulation two homolog 1) promotes PGC-1α activity (37, 38). The mechanistic inter-relationships among these post-translational modifications are incompletely understood. We hypothesize that PGC-1α might be an important regulator of Ang II-induced vascular hypertrophy through a mechanism that depends on post-translational modifications of PGC-1α.

We previously reported that Ang II-induced activation of Akt is mediated through rapidly induced increases in intracellular H_2O_2 (8). Here we show that Ang II stimulation inhibits transcriptional activities of PGC-1α via Akt-mediated phosphorylation at Ser^{570}. This phosphorylation is required for the binding of GCN5 to and the subsequent lysine acetylation of PGC-1α. These sequential events result in disruption of the PGC-1α·FoxO1 complex binding to the FoxO1 response element of the catalase promoter, thereby down-regulating catalase expression and increasing ROS levels and hypertrophy in VSMCs. These findings extend understanding of the functional consequences of Akt-mediated PGC-1α serine 570 phosphorylation by revealing its enabling role in GCN5-mediated lysine acetylation. These findings provide insights into a novel mechanism by which Ang II may mediate its disparate maladaptive pathophysiologic and metabolic functions through interactive post-translational modifications of PGC-1α.

EXPERIMENTAL PROCEDURES

Materials—Antibodies to PGC-1α (H-300), GCN5 (H-75), FKHR/FoxO1 (H-128), Histone H1 (FL-219), and Akt1/2 (H-136) were from Santa Cruz. Rabbit polyclonal PGC-1α antibody was a gift from Dr. D. Kelly. Mouse monoclonal antibody to GCN5 (ab61174) was from Abcam Inc. Anti-catalase rabbit polyclonal antibody was from Calbiochem. Antibodies to phosphoserine (PSR-45), tubulin (T5168), and actin (20–33) were from Sigma. Antibodies to acetylated-lysine (9681), phospho-Akt (Ser^{473}), phospho-p38 MAPK (Thr^{180}/Tyr^{182}), phospho-p44/42 (Thr^{202}/Tyr^{204}), p38 MAPK (9212), and p44/42 MAPK (9102) were from Cell Signaling. H_2-dichlorofluorescin diacetate (DCFDA) was from Molecular Probes. LY294002 was from Alexis Corp. SB203580, SP600125, PD98059, and the Akt inhibitor V/triciribine were purchased from Calbiochem. The nuclear/cytosol fractionation kit (K266-100) was from BioVision. Expression plasmids for Gal4-PGC-1α and Gal4 responsive reporter plasmid pGal4-FFL were purchased from Addgene. [^{3}H]Leucine (140 Ci/mmol) was from PerkinElmer Life Sciences. ECL Western blotting detection reagents and nitrocellulose membranes (Hybond-ECL) were obtained from Amersham Biosciences. A basic Nucleofector® kit for primary smooth muscle cells was from Biosystems. All other chemicals and reagents, including Ang II, catalase-polyethylene glycol, actinomycin D, and Dulbecco's modified Eagle's medium (DMEM) with 25 mM Hepes and 4.5 g/liter glucose were from Sigma.

Cell Culture and Adenovirus Transduction—VSMCs were isolated from male Sprague-Dawley rat thoracic aortas by enzymatic digestion as described previously (39). The cells were grown in DMEM supplemented with 10% calf serum, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin and were passaged twice a week by harvesting with trypsin:EDTA and seeding into 75-cm^2 flasks. For experiments, the cells between passages 6 and 15 were used at confluence. VSMCs were growth-arrested for 24 h prior to treatment with 100 nM Ang II. For adenovirus-mediated protein overexpression, VSMCs were infected with various multiplicities of infection of either Ad.PGC-1α (a gift from Dr. B. Spiegelman), Ad.PGC-1α S570A mutant (a gift from Dr. M. Birnbaum), Ad.Akt-dn (40), or control adenovirus (Ad.LacZ) encoding β-galactosidase (a gift from Dr. B. Berk) for 1 h in serum-free medium. Then the virus was removed, and the cells were cultured for an additional 24–48 h before Ang II stimulation. The multiplicity of infection (ratio of plaque-forming units:colony-forming units) was calculated by using optical density (41, 42).

Subcellular Fractionation—The nuclear and cytoplasmic extracts from VSMCs were prepared using a nuclear/cytosol fractionation kit (K266-100) according to the manufacturer's instructions. Equal amounts of total cell lysates, nuclear extracts, or cytoplasmic extracts were subjected to immunoblotting. The separation between the nuclear and cytosolic fractions was verified by blotting for nuclear protein histone H1 and cytosolic protein tubulin.

Western Blotting and Immunoprecipitation—VSMCs were lysed in lysis buffer (50 mM Hepes, pH 7.4, 50 mM NaCl, 1% Triton X-100, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 mM sodium pyrophosphate, 50 mM sodium fluoride, and 1 mM sodium orthovanadate. The lysates were resolved by SDS-PAGE and immunoblotted with appropriate antibodies. For immunoprecipitation, growth-arrested VSMCs were stimulated with Ang II at 37 °C, and 100–500 μg of nuclear extracts were immunoprecipitated with PGC-1α antibodies and protein A/G Plus-agarose beads (Santa Cruz). The samples were separated by SDS-PAGE, transferred to nitrocellulose membranes, and incubated overnight with indicated antibodies. After incubation with secondary antibodies, the proteins were detected by enhanced chemiluminescence.

Chromatin Immunoprecipitation (ChIP) Assay—ChIP assay was performed according to the manufacturer's instructions (Upstate Biotechnology Inc.). VSMCs were grown to 60–80% confluence in DMEM supplemented with 10% calf serum. Thereafter, the cells were starved for 24 h. Following treatment

with 100 nM Ang II for the indicated times, the cells were fixed with 1% formaldehyde at 37 °C for 10 min, lysed, and sonicated. Soluble chromatin was coimmunoprecipitated with anti-PGC-1α or anti-FKHR/FoxO1 antiserum or an equal amount of control rabbit IgG. The cells at ~80% confluency grown in one 10-cm diameter dish were used per immunoprecipitation. After de-cross-linking of the DNAs, DNA purified from starting (1% input) and immunoprecipitated samples was subjected to PCR using the following primers: catalase FoxO1-DBE (−2160 to −1938), 5′-GGCTTCTGTTTGCCTTGCTCAG-3′ and 5′-ATACTGATACTGCGATTGTTAAGCG-3′. The region of amplification contains FoxO1-dependent binding element (FoxO1-DBE) in catalase promoter (NC_005102.2). Standard PCRs were performed. The GAPDH promoter was analyzed by ChIP in parallel as a control. The relative ChIP units were calculated by the ratio of ChIP DNA to input DNA using densitometry.

Luciferase Reporter Gene Assay—Cells in 12-well plates were cotransfected with the Gal4-responsive reporter construct (100 ng), expression vector encoding Gal4-PGC-1α (400 ng), the empty vector (pM, 400 ng), and the internal control p-RL-TK vector (50 ng) using the Basic Nucleofector® Amaxa transfection kit (Biosystems). When necessary, inhibitors were added for 30 min prior to Ang II stimulation. Various doses of Ang II were added to cells for another 16 h. The cells were harvested, and luciferase activity was determined using a dual luciferase reporter assay system (Promega) according to the manufacturer's instructions. The relative activity was calculated as the ratio of firefly luciferase activity to *Renilla* luciferase activity (internal control). The data from three independent experiments (each performed in triplicate) are shown as the averages ± the standard error of the mean.

Reverse Transcription (RT)-PCR—Total RNA was extracted from VSMCs using TRIzol® reagent (Invitrogen) and treated with DNase I according to the manufacturer's instructions. The concentration and purity of RNA were determined by spectrophotometer at 260 and 280 nm. RT-PCR amplification was performed using a SuperScript™ one-step RT-PCR system with Platinum *Taq* DNA polymerase (Invitrogen). One microgram of total RNA was used as a template for subsequent RT and PCRs. The cDNA was amplified with the following PCR primers: sense 5′-GTGGTTTTCACCGACGAGAT-3′ and antisense 5′-CATGTCAGGGTCCTTCAGGT-3′ for rat catalase (NP_036652); sense 5′-AAACTTGCTAGCGGTCCTCA-3′ and antisense 5′-ACGTCTTTGTGGCTTTTGCT-3′ for rat PGC-1α (NP_112637); and sense 5′-GGTGATGCTGGTGCTGAGTA-3′ and antisense 5′-ACTGTGGTCATGAGCCCTTC-3′ for rat GAPDH (NM_017008). GAPDH served as an internal control of the reaction. The PCR cycling protocol consisted of 30 cycles of denaturation at 94 °C for 2 min, annealing at 54 °C for 30 s, and extension at 72 °C for 1 min, followed by final extension at 72 °C for 5 min. PCR products were analyzed by electrophoresis on 1.8% agarose gels containing ethidium bromide.

mRNA Stability of Rat Catalase in VSMCs—Half-lives of catalase mRNA in VSMCs with or without Ang II pretreatment were calculated by linear regression analysis of the rate of catalase mRNA decay as a function of time after actinomycin D (5 μg/ml) administration. Total RNA was isolated from the cells at the times indicated, and the mRNA levels of catalase and housekeeping gene-GAPDH were semi-quantified by RT-PCR. The normalized mean data from four separate experiments were plotted on logarithmic scales using GraphPad Prism. The half-lives were calculated as the time required for each mRNA decreasing to 50% of its initial abundance.

RNA Interference—Sequences of custom siRNA duplex for rat FoxO1 (Applied Biosystem; Ambion) were as follows: sense 5′-CUGUGCGCCUGGACUCUUG(dTdT)-3′ and antisense 5′-CAAGAGUCCAGGCGCACAG(dTdT)-3′. The target sequences for rat GCN5 were as follows: sense 5′-UGUUCGAGCUCUCAAAGAU(dTdT)-3′ and antisense 5′-AUCUUUGAGAGCUCGAACA(dTdT)-3′. The target sequences for rat PGC-1α siRNA duplex were as follows: sense 5′-GAUUCAAACUCAGACGAUU(dTdT)-3′ and antisense 5′-AAUCGUCUGAGUUUGAAUC(dTdA)-3′. The scrambled siRNA duplex controls used were designed and supplied by Ambion. The target sequences for rat catalase siRNA duplex (Invitrogen) were as follows: sense 5′-UGGCUAUGGCUCACACACC(dTdT)-3′ and antisense 5′-GGUGUGUGAGCCAUAGCCA(dTdT)-3′. A nonrelated scrambled RNAi (5′-CACUGACAGUCUGACUCCG(dTdT)-3′) without any other match in the rat genomic sequence was used as a control. Transfection of siRNA duplexes into VSMCs was performed according to the manufacturer's instructions of the basic Nucleofector® kit for primary smooth muscle cells (Amaxa Biosystem). The efficiency and specificity of siRNA depletion were verified by Western blot using the specific antibodies.

Intracellular H_2O_2 Measurement—VSMCs were plated at low density, grown for 2 days in culture medium containing 10% calf serum, and growth-arrested for another day in culture medium containing 0.1% calf serum. The cells were then stimulated with 100 nM Ang II for 24 h. The intracellular ROS levels were determined using 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA; Molecular Probes). For assays, the cells were incubated with 10 μM H_2DCFDA in Hanks' balanced salt solution/calcium/magnesium buffer for 30 min. The cells were trypsinized, washed, and resuspended in Hanks' balanced salt solution. Dichlorofluorescein fluorescence was immediately measured by a CytoFluor multi-well plate reader.

[^{3}H]Leucine Incorporation—To measure hypertrophy of VSMCs, the cells were quiesced for 48 h in DMEM containing 0.1% calf serum. Twenty-four hours before harvest, the cells were incubated with [^{3}H]leucine (1 μCi/ml) in the presence or absence of 100 nM Ang II for 24 h. The cells were washed twice with phosphate-buffered saline, incubated with 5% trichloroacetic acid for 5 min on ice, and lysed with 0.4 M NaOH. Scintillation fluid was applied to the lysates, and the mixtures were counted in a liquid scintillation counter.

Statistical Analysis—All of the values were expressed as the means ± S.E. The data were analyzed by Student's *t* test or analysis of variance of the repeated experiments, followed by the Tukey's *post hoc* test when appropriate with Prism software (GraphPad Software). Statistical significance was accepted at $p < 0.05$.

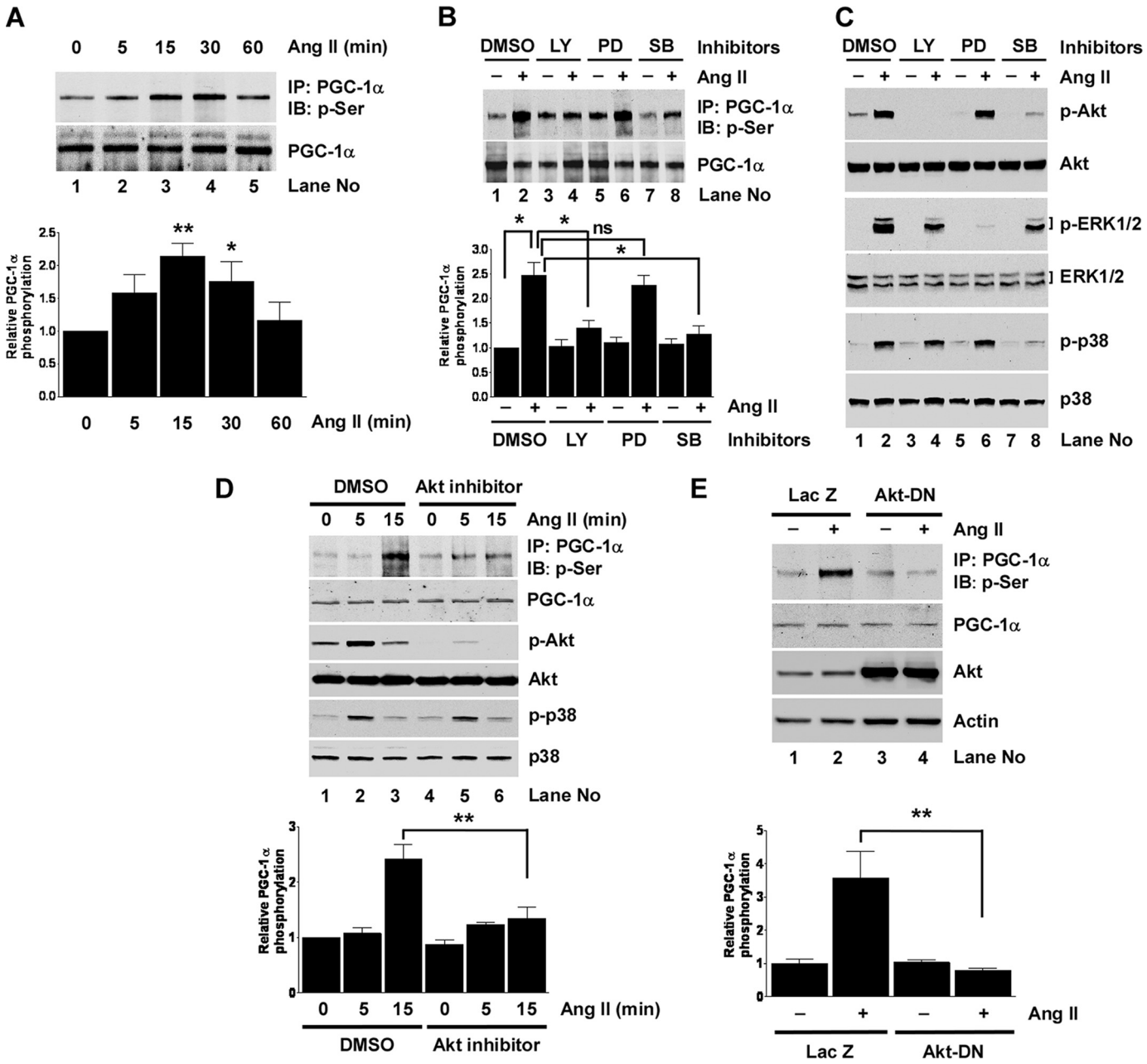

FIGURE 1. **Ang II induces Akt-dependent PGC-1α phosphorylation in VSMCs.** *A*, growth-arrested VSMCs were stimulated with 100 nM Ang II for the indicated times. *B–D*, VSMCs were pretreated with 10 μM LY294002, 10 μM SB203580, or 10 μM PD98059 (*B* and *C*) or with 1 μM Akt inhibitor V/triciribine (*D*) for 30 min prior to treatment with (+) or without (−) 100 nM Ang II for 15 min (*B* and *C*) or for the indicated times (*D*). *E*, VSMCs were infected with the adenovirus encoding a dominant negative mutant Akt/PKB (Ad.Akt-DN) or a control adenovirus Ad.Lac Z, and starved for an additional 24 h before Ang II stimulation for 15 min. Phosphorylation of Akt, ERK1/2, and p38-MAPK was detected using the specific antibodies (*C*). The membranes were stripped and reprobed with antibodies against nonphosphorylated proteins. Nuclear extracts were immunoprecipitated (*IP*) with PGC-1α antibody followed by blotting with phosphoserine antibody (*A*, *B*, *D*, and *E*). Immunoblotting (*IB*) with PGC-1α is shown as a loading control. The bar graphs represent the averaged data (means ± S.E.) for levels of PGC-1α phosphorylation ($n = 3–5$), corrected for total PGC-1α loading, expressed as fold change over basal. *, $p < 0.05$; **, $p < 0.01$; *ns*, no significant difference.

RESULTS

Ang II Induces Serine Phosphorylation of PGC-1α through Akt in VSMCs—We first examined whether Ang II induces PGC-1α phosphorylation. Because PGC-1α localizes mainly in the nucleus (supplemental Fig. S1), we used nuclear extracts to immunoprecipitate PGC-1α and determine its phosphorylation by Western blot. Ang II (100 nM) stimulation increased serine phosphorylation of PGC-1α in a time-dependent manner without affecting PGC-1α protein expression (Fig. 1*A*). Phosphorylation of PGC-1α reached peak levels by 15 min after Ang II stimulation and gradually decreased by 60 min, providing evidence that PGC-1α is an Ang II target. Our group and others have shown that Ang II stimulates phosphorylation of Akt, p38 MAPK, and ERK1/2 after 5 min of Ang II treatment (supplemental Fig. S2). These three kinases are involved in Ang II-induced hypertrophy. To investigate the upstream kinases mediating PGC-1α phosphorylation by Ang II, we incubated cells with specific kinase inhibitors before the addition of Ang

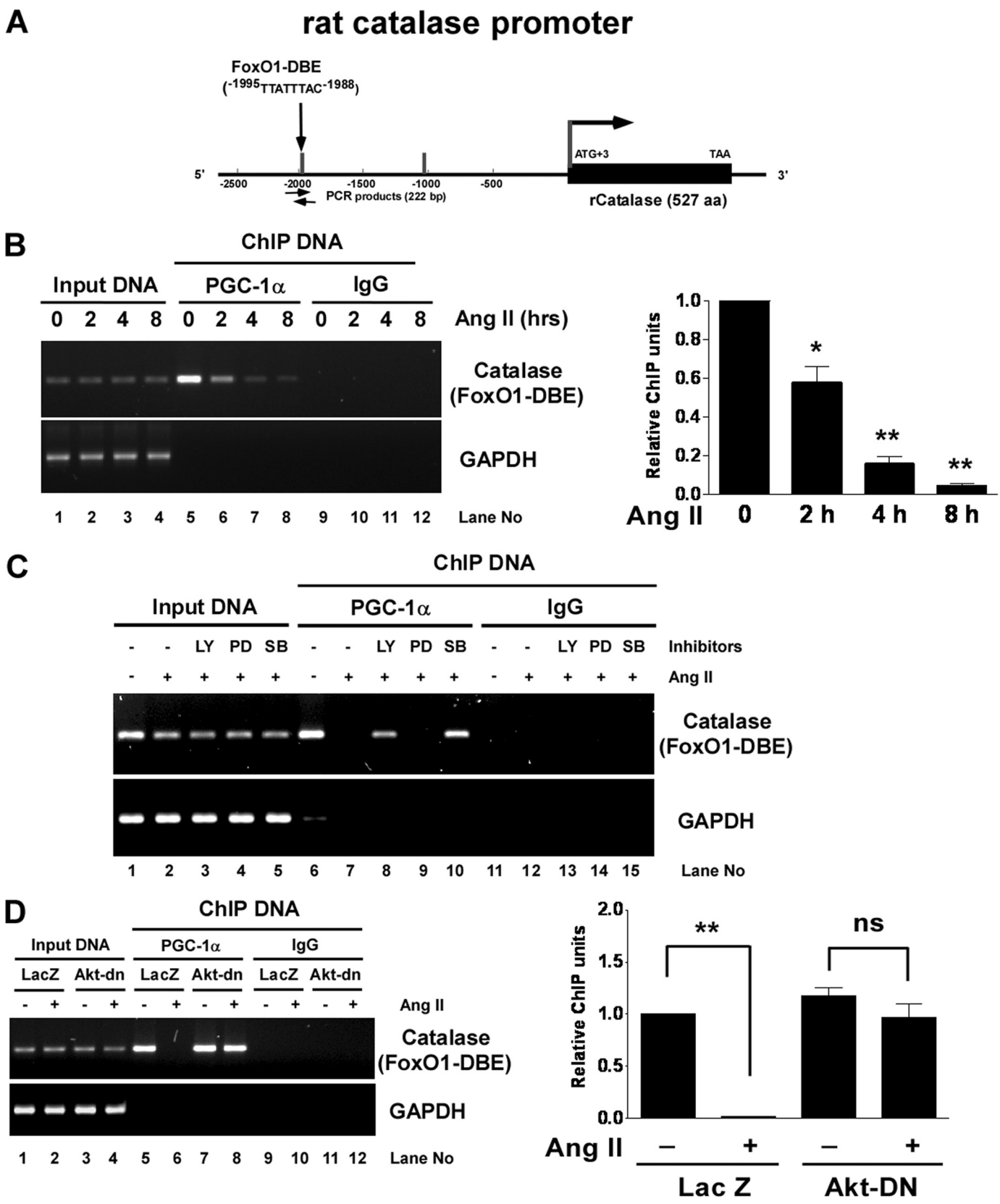

FIGURE 2. **Ang II induces Akt-dependent PGC-1α dissociation from the catalase promoter.** *A*, catalase promoter contains a conserved FoxO1-DBE: −1995TTATTTAC-1988. *B*, growth-arrested VSMCs were stimulated for the indicated times with 100 nM Ang II. *C*, VSMCs were pretreated with 10 μM LY294002, 10 μM PD98059, or 10 μM SB203580 for 30 min prior to treatment with 100 nM Ang II for 8 h. *D*, VSMCs were infected with Ad-HA-Akt(AAA) or Ad.Lac Z (control) and stimulated with Ang II for 8 h. ChIP assay was performed as described under "Experimental Procedures." The result of one representative experiment is shown. The bar graphs represent averaged data (means ± S.E.) for PGC-1α binding to the catalase promoter ($n = 3–5$), expressed as fold change over basal. The relative ChIP units were calculated by the ratio of ChIP DNA to input DNA using densitometry. *, $p < 0.05$; **, $p < 0.01$ *versus* no Ang II treatment; *ns*, no significant difference.

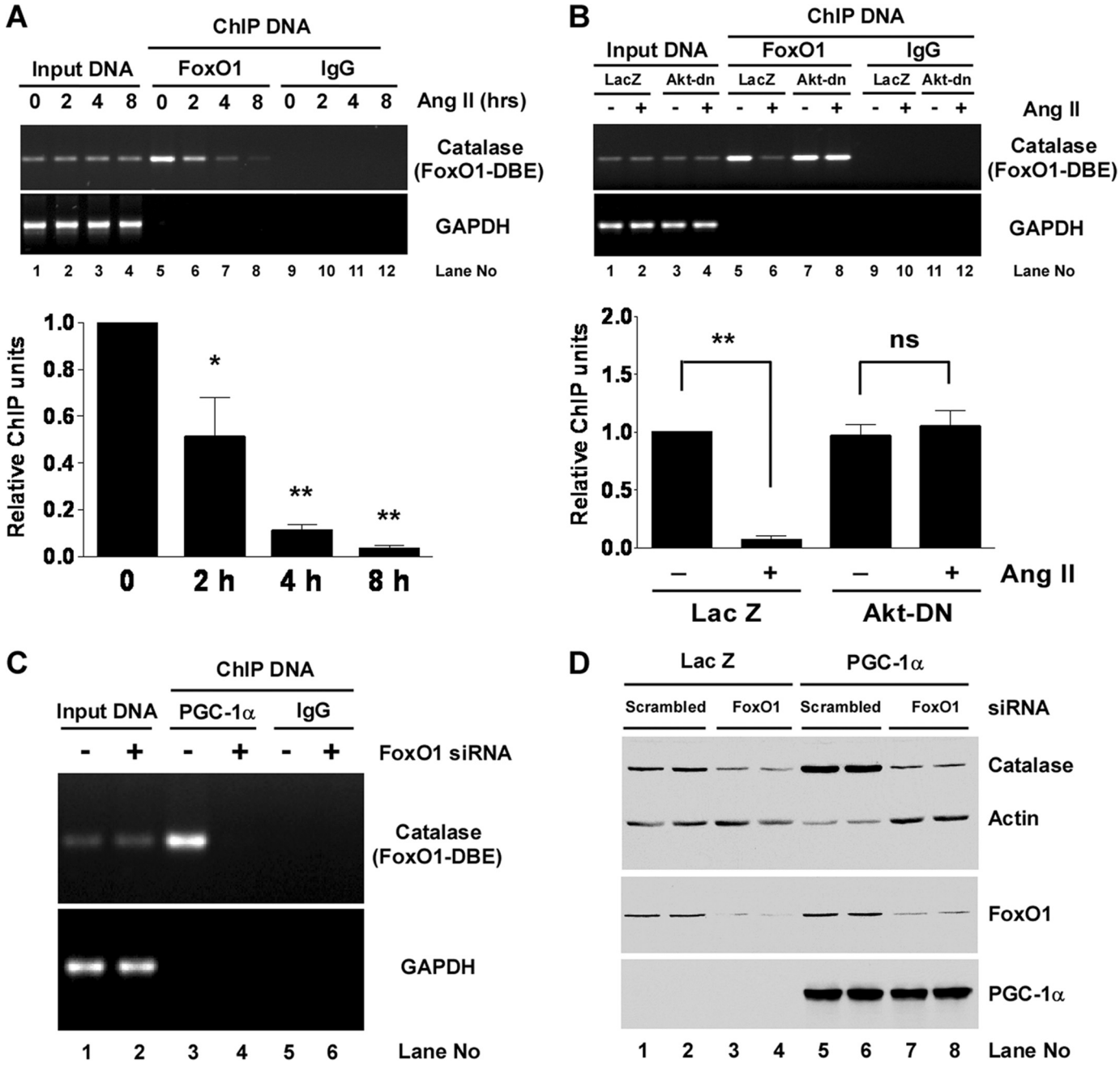

FIGURE 3. **FoxO1 is required for PGC-1α-mediated catalase expression.** *A* and *B*, Ang II induces Akt-dependent FoxO1 dissociation from the catalase promoter. *A*, VSMCs were stimulated for the indicated times with 100 nM Ang II. *B*, VSMCs were infected with Ad-HA-Akt(AAA) or Ad.Lac Z (control) and stimulated with Ang II for 4 h. ChIP assay was performed as described under "Experimental Procedures." The bar graphs represent the averaged data (means ± S.E.) for FoxO1 binding to the catalase promoter ($n = 3$), expressed as fold change over basal. *C* and *D*, FoxO1 is required for PGC-1α binding to the catalase promoter and PGC-1α-coactivated catalase expression. VSMCs were transfected with 30 nM FoxO1 siRNA or control scrambled siRNA for 48 h; the cells were then infected with the adenovirus encoding wild type PGC-1α or lac Z for 24 h. ChIP assay for PGC-1α binding to the catalase promoter (*C*) was performed as described under "Experimental Procedures." Protein expression (*D*) for catalase, actin (loading control), FoxO1, and PGC-1α was analyzed by Western blot. One representative result of three separate experiments is shown.

II. Inhibitors of both PI3K/Akt (LY294002, 10 μM) and p38 MAPK (SB203580, 10 μM) significantly reduced Ang II-stimulated PGC-1α serine phosphorylation (Fig. 1*B*, compare *lanes 4* and *8* with *lane 2*). In contrast, the ERK1/2 inhibitor (PD98059) had no effect. Under the same conditions, inhibition of p38 MAPK by SB203580 also blocked Ang II-induced Akt serine 473 phosphorylation (Fig. 1*C*, *lanes 7* and *8*), as previously described (43–47), whereas inhibition of Akt activation by LY294002 did not affect Ang II-induced p38 MAPK phosphorylation. These results suggest that p38 MAPK is upstream of Akt in VSMCs. Of note, Ang II-induced PGC-1α phosphorylation was significantly blocked by a specific Akt inhibitor V (Fig. 1*D*) and by overexpression of a dominant negative Akt (DN-Akt) mutant (Fig. 1*E*). These results suggest that p38 MAPK and Akt may act on the same signaling axis to mediate PGC-1α serine phosphorylation by Ang II in VSMCs.

Ang II Inhibits PGC-1α Cotranscriptional Activity and Disrupts Endogenous PGC-1α Association with the Catalase

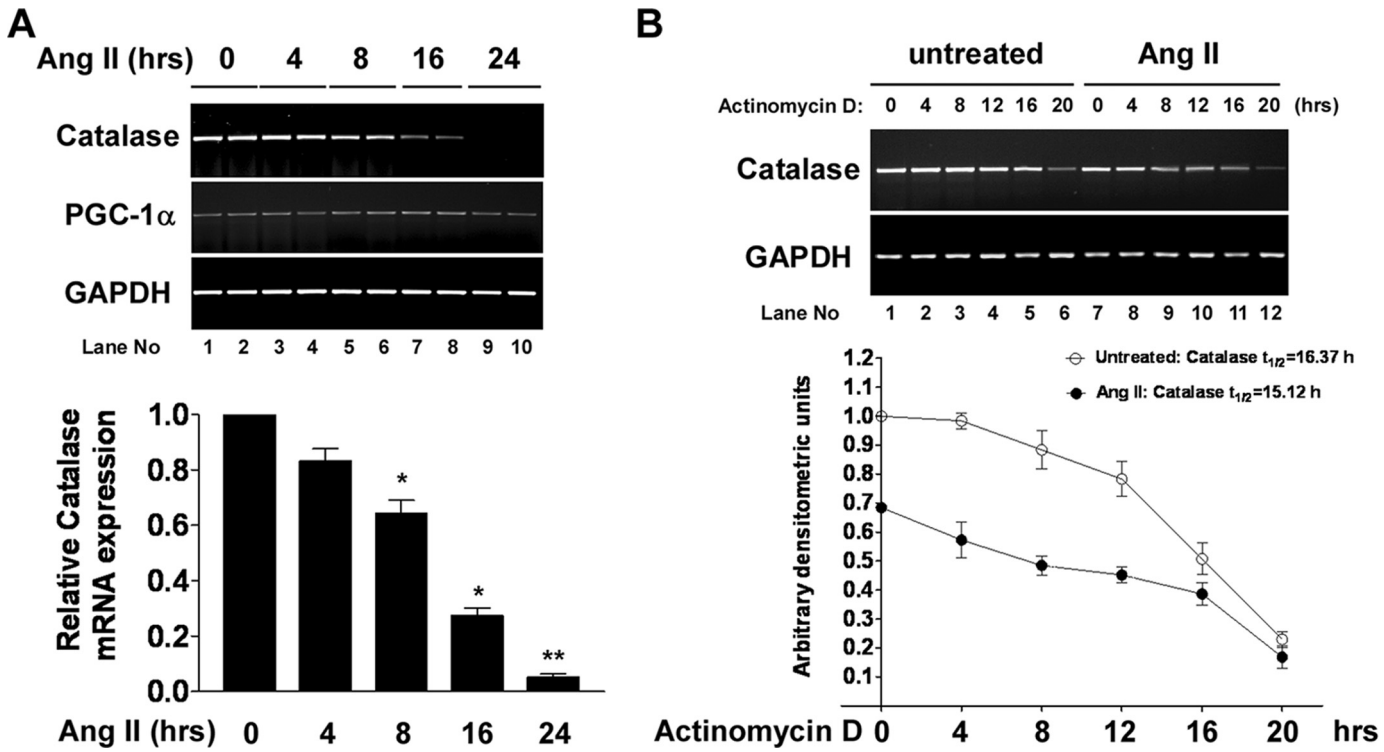

FIGURE 4. **Effect of Ang II on catalase mRNA level and stability in VSMCs.** *A*, growth-arrested VSMCs were stimulated with 100 nM Ang II for the indicated times. Messenger RNA was prepared and assayed for catalase, PGC-1α, and GAPDH levels by RT-PCR ($n = 6$). The result of one representative experiment is shown. The bar graphs represent the averaged data (means ± S.E.) for catalase expression ($n = 3$–5), expressed as fold change over basal. *, $p < 0.05$; **, $p < 0.01$; *ns*, no significant difference. *B*, growth-arrested VSMCs were pretreated for 8 h with 100 nM Ang II or vehicle before 5 μg/ml actinomycin D was added to block transcription. Total RNA was extracted from untreated and Ang II-treated cell cultures incubated with actinomycin D at the times indicated, and catalase and GAPDH mRNA was semiquantified by RT-PCR. Representative PCR products visualized in ethidium bromide-stained agarose gels are shown; the levels of GAPDH served to verify equal sample input. The normalized mean data from four separate experiments were plotted on logarithmic scales using GraphPad Prism. Half-life of catalase mRNA was calculated as the time required for each mRNA decrease to 50% of its initial abundance.

Promoter—Because PGC-1α cotranscriptional activity is regulated by phosphorylation, we employed fusion constructs Gal4-PGC-1α and Gal4-responsive reporter-pGal4-FFL and performed reporter transfection assays to determine the effect of Ang II on PGC-1α cotranscriptional activity in VSMCs. The intrinsic PGC-1α cotranscription activity was significantly reduced by Ang II in a dose-dependent manner (supplemental Fig. S3*A*). Ang II-induced inhibition of PGC-1α cotranscriptional activity was partially but significantly reversed by PI3K or p38 MAPK inhibitor, but not by the ERK1/2 inhibitor (supplemental Fig. S3*B*), suggesting that Ang II-induced Akt phosphorylation may suppress PGC-1α cotranscriptional activity in VSMCs.

To gain insight into the role of PGC-1α in ROS-dependent Ang II signaling, we tested whether PGC-1α regulates catalase expression. Firstly, we analyzed the promoter region of the rat catalase gene (NM_012520) and found that it contains a FoxO1-dependent DBE (TTATTTAC) (48) located at nucleotides −1995 to −1988 (Fig. 2*A*). As noted, FoxO1 is coactivated by PGC-1α (28). ChIP assays using specific PGC-1α antibody revealed that endogenous PGC-1α bound to the catalase promoter region encompassing FoxO1-DBE in basal state *in vivo* (Fig. 2*B*, *lane 5*). Ang II treatment decreased PGC-1α binding to the catalase promoter in a time-dependent manner, which occurred within 2 h and continued to decrease for over 8 h (Fig. 2*B*). The reduced PGC-1α binding to the catalase promoter by Ang II was prevented by PI3K and p38 MAPK inhibitors (Fig. 2*C*, *lanes 8* and *10*, respectively) as well as by overexpression of DN-Akt (Fig. 2*D*, *lane 8*). ERK1/2 inhibition had no effect (Fig. 2*C*, *lane 9*). Thus, endogenous PGC-1α binds to the FoxO1-DBE of the catalase promoter basally, and Ang II stimulation disrupts its binding to the promoter in an Akt-dependent manner.

FoxO1 Is Required for PGC-1α-mediated Catalase Expression in VSMCs—We next assessed whether FoxO1 could mediate PGC-1α binding to FoxO1-DBE of the catalase promoter. ChIP assay (Fig. 3*A*) showed that FoxO1 bound to the catalase promoter, which was disrupted after 2 h of Ang II treatment similar to PGC-1α (compare Fig. 3*A* with Fig. 2*B*). Furthermore, Ang II-induced reduction of FoxO1 binding to the catalase promoter was prevented by overexpression of DN-Akt (Fig. 3*B*, compare *lane 8* with *lane 6*). To test whether FoxO1 mediates the association of PGC-1α with catalase promoter, we examined the effects of FoxO1 depletion using siRNA. ChIP showed that PGC-1α binding to catalase promoter was completely abolished by the FoxO1 siRNA (Fig. 3*C*). Increasing evidence showed that PGC-1α binds to FoxO1 and coactivates FoxO1-dependent gene expression (28–30). These results suggest that Ang II treatment dissociates PGC-1α·FoxO1 complex from catalase FoxO1-DBE to down-regulate catalase expression. We thus determined the impact of FoxO1 depletion on PGC-1α-dependent catalase expression analyzed by Western blot (Fig. 3*D*). FoxO1 depletion by siRNA decreased catalase protein expression, whereas overexpression of PGC-1α in-

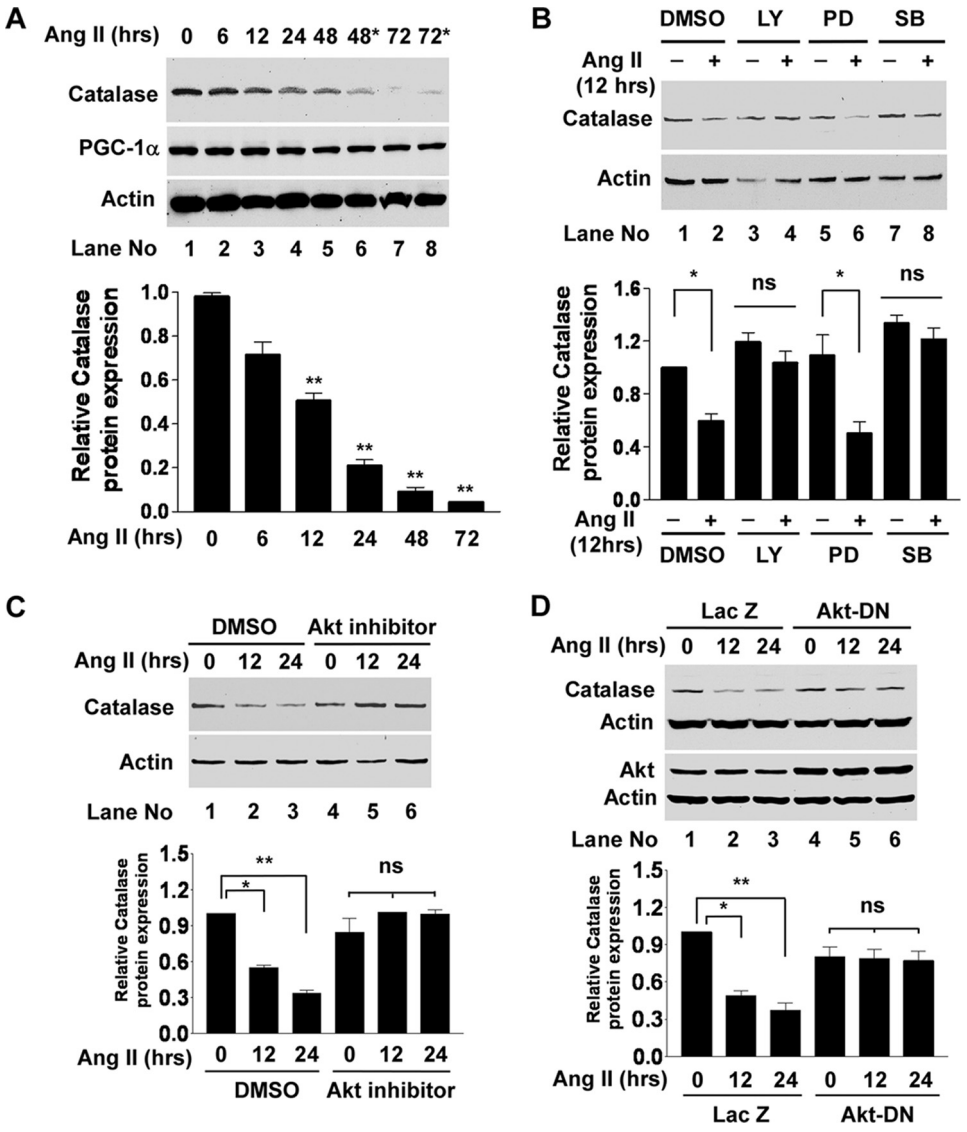

FIGURE 5. **Ang II decreases endogenous catalase protein expression through Akt-dependent manner.** *A*, growth-arrested VSMCs were stimulated with 100 nM Ang II for the indicated times. Protein expression was analyzed by Western blot with indicated antibodies. *48** and *72** denote that Ang II was added into medium again every 24 h. *B–D*, VSMCs were pretreated with 10 μM PD98059 (*PD*), LY294002 (*LY*), or SB203580 (*SB*, *B*) or with 1 μM Akt inhibitor V/triciribine (*C*) or infected with Ad.Akt-DN or Ad.Lac Z (control) (*D*) prior to stimulation with 100 nM Ang II for the indicated times. The result of one representative experiment is shown. The bar graphs represent the averaged data (means ± S.E.) for catalase expression ($n = 3–5$), expressed as fold change over basal. *, $p < 0.05$; **, $p < 0.01$; *ns*, no significant difference. *DMSO*, dimethyl sulfoxide.

creased it. Moreover, FoxO1 depletion prevented and further reduced PGC-1α-activated catalase expression to similar levels of those observed in FoxO1 siRNA alone (Fig. 3*D*, compare *lanes 7* and *8* with *lanes 3* and *4*). These data indicate that FoxO1 is necessary and sufficient for PGC-1α-dependent regulation of catalase expression.

Ang II Reduces Catalase Expression in an Akt-dependent Manner—We assessed the functional consequences of Ang II-induced inhibition of PGC-1α·FoxO1 association with the catalase promoter by determining catalase mRNA and protein levels over time. Ang II significantly decreased catalase mRNA levels after 8 h of treatment; the levels were almost undetectable by 24 h (Fig. 4*A*). No changes were observed in PGC-1α or GAPDH mRNA. Strikingly, PGC-1α, as well as FoxO1 association with the catalase promoter, was reduced more than 80% after 4 h of Ang II stimulation (Figs. 2*B* and 3*A*, respectively). To investigate the apparent discrepancy between PGC-1α·FoxO1 loss at the promoter and catalase mRNA levels, we measured catalase mRNA half-life ($t_{1/2}$) in the presence of actinomycin D (Fig. 4*B*). The half-life for catalase mRNA in the absence of Ang II stimulation was about 16 h. After 8 h of exposure to Ang II before the addition of actinomycin D, the $t_{1/2}$ was also ~16 h. Thus, catalase mRNA has a rather prolonged half-life that was not importantly changed by Ang II. The $t_{1/2}$ of the mRNA is consistent with the time course of Ang II-induced reduction of catalase protein at 12–24 h as shown in Fig. 5*A*.

Ang II-induced down-regulation of catalase expression was prevented by PI3K or p38 MAPK but not ERK1/ERK2 inhibitors (Fig. 5*B*) as well as by Akt inhibitor (Fig. 5*C*) or DN-Akt overexpression (Fig. 5*D*). These results are consistent with the notion that Ang II-induced, Akt-mediated dissociation of the PGC-1α·FoxO1 complex from the catalase promoter may contribute to the reduction in catalase expression in VSMCs.

Phosphorylation of PGC-1α at Serine 570 Is Required for Ang II-induced Catalase Down-regulation—Cotranscriptional activity of PGC-1α regulating gluconeogenesis and fatty acid oxidation was shown to be suppressed by phosphorylation at serine 570 by Akt, which leads to inhibition of PGC-1α association with the cognate promoter (34). Thus, we examined whether serine 570 phosphorylation of PGC-1α is involved in Ang II-induced decreased association of PGC-1α with the catalase promoter and reduced catalase expression. Ang II-induced serine phosphorylation of PGC-1α was almost completely abolished by overexpression of PGC-1α (S570A) mutant but was significantly enhanced by PGC-1α (wt) (Fig. 6*A*). Thus, serine 570 is likely to be a major Ang II-induced PGC-1α serine phosphorylation site. Overexpression of the PGC-1α (S570A) mutant, but not PGC-1α (wt), prevented both the Ang II-induced dissociation of PGC-1α from the catalase promoter (Fig. 6*B*, compare *lane 10* with *lane 12*) and the decrease in catalase protein expression (Fig. 6*C*). Both PGC-1α (wt) and the S570A mutant showed substantial basal association with the catalase promoter containing FoxO1-DBE (Fig. 6*B*) and increased basal catalase protein

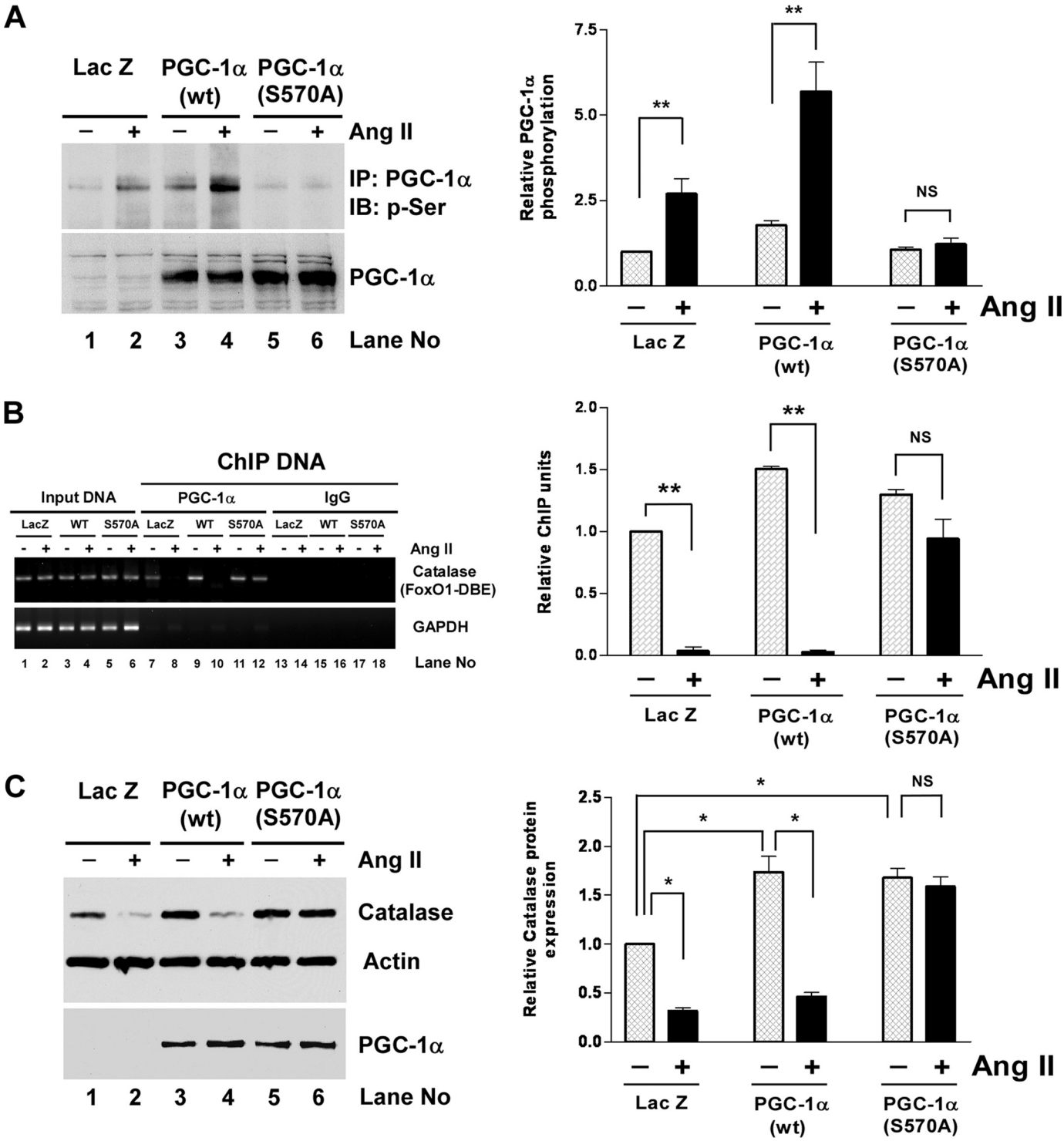

FIGURE 6. **Akt-dependent PGC-1α serine 570 phosphorylation is required for Ang II-induced catalase down-regulation.** VSMCs in serum-free DMEM were infected with the adenovirus encoding wild type PGC-1α (Ad.PGC-1α (wt)), mutant PGC-1α (S570A) (Ad.PGC-1α(S570A)), or Ad.Lac Z (control) for 24 h. The cells were stimulated with Ang II either for 15 min for analysis of PGC-1α phosphorylation (*A*), for 8 h for PGC-1α binding to the catalase promoter using ChIP assay (*B*), or for 24 h for protein expression for catalase, actin (loading control), and PGC-1α (*C*). The bar graphs represent averaged data (means ± S.E.), expressed as fold change over basal ($n = 5$). *, $p < 0.05$; **, $p < 0.01$ between the indicated conditions; *ns*, no significant difference; *IP*, immunoprecipitation; *IB*, immunoblot.

expression (Fig. 6*C*), compared with nontransfected VSMCs. Serine 570 phosphorylation of PGC-1α is required for Ang II-induced catalase down-regulation probably by preventing PGC-1α associating with the catalase promoter. Thus, Akt-mediated serine 570 phosphorylation is necessary for Ang II-induced dissociation of PGC-1α from the catalase promoter.

PGC-1α Phosphorylation at Serine 570 Is Required for Subsequent Acetylation by GCN5—Multiple post-translational modifications including phosphorylation and acetylation modulate

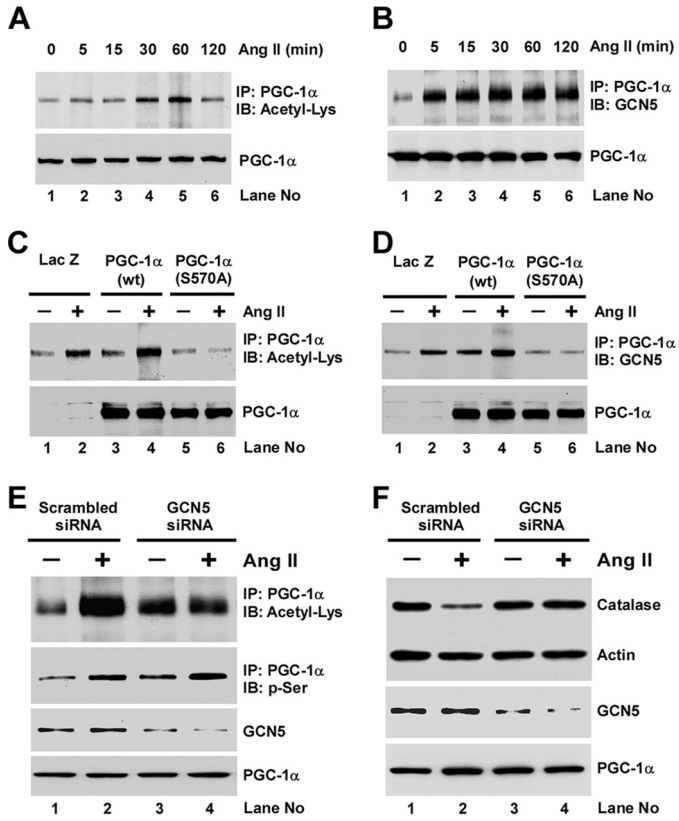

FIGURE 7. **Sequential serine 570 phosphorylation and GCN5-mediated acetylation of PGC-1α are required for Ang II-induced catalase down-regulation.** *A* and *B*, VSMCs were stimulated with Ang II for the indicated times. Nuclear extracts were immunoprecipitated with PGC-1α antibody followed by blotting with acetylated-lysine (*A*) or GCN5 (*B*) antibodies. Immunoblotting with PGC-1α is shown as a loading control. *C* and *D*, VSMCs were infected with the adenovirus encoding wild type PGC-1α (Ad.PGC-1α (*wt*)), mutant PGC-1α (S570A)(Ad.PGC-1α(S570A)), or Ad.Lac Z (control) for 24 h. The cells were stimulated with Ang II for 30 min for analysis of PGC-1α acetylation (*C*) and interaction of PGC-1α with GCN5 (*D*). *E* and *F*, VSMCs were transfected with 30 nM GCN5 siRNA or control scrambled siRNA for 48 h. The cells were starved and stimulated with Ang II for 30 min for analysis of PGC-1α acetylation and serine phosphorylation by immunoprecipitation (*E*) and for 24 h for analysis of catalase expression by Western blot (*F*). The data shown are from one of three or four independent experiments. *IP*, immunoprecipitation; *IB*, immunoblot.

PGC-1α activity. GCN5, a histone acetyltransferase, was identified as a specific endogenous repressor of PGC-1α. GCN5 directly acetylates and removes PGC-1α from promoter regions to nuclear foci to control glucose metabolism (36). We posited that phosphorylation of PGC-1α serine 570 could regulate its function by modulating its acetylation. To test this hypothesis, we first examined whether Ang II affects PGC-1α acetylation status. As shown in Fig. 7*A*, Ang II treatment promoted lysine acetylation of PGC-1α in a time-dependent manner without affecting PGC-1α protein expression. Acetylation of PGC-1α reached peak levels by 60 min after Ang II stimulation and gradually decreased by 120 min. Quantitation of increased acetylation levels of PGC-1α by Ang II is presented in supplemental Fig. S4. Furthermore, Ang II promotes a sustained interaction of PGC-1α with GCN5 (Fig. 7*B*) after 5 min of treatment. These results infer that agonist-induced GCN5 binding is important in PGC-1α acetylation and inhibition of its transcriptional activity. Also, inhibition of serine 570 phosphorylation by overexpression of PGC-1α (S570A) mutant essentially abolished Ang II-induced PGC-1α acetylation (Fig. 7*C*, compare *lane 6* with *lane 2*) and its association with GCN5 (Fig. 7*D*, compare *lane 6* with *lane 2*). Finally, we examined whether GCN5-mediated PGC-1α lysine acetylation might be responsible for catalase down-regulation by Ang II. To this end, we knocked down endogenous GCN5 by RNA interference. GCN5 depletion prevented Ang II-induced PGC-1α acetylation (Fig. 7*E*, compare *lane 3* with *lane 4*). GCN5 depletion, on the other hand, did not inhibit Ang II-induced PGC-1α serine 570 phosphorylation (Fig. 7*E*, compare *lanes 1* and *2* with *lanes 3* and *4*). Consistently, inhibition of PGC-1α acetylation by GCN5 depletion prevented Ang II-induced down-regulation of catalase expression (Fig. 7*F*, compare *lane 3* with *lane 4*). GCN5 knockdown, however, increased basal levels of acetylation of PGC-1α, suggesting that in the absence of GCN5, other acetyltransferases may acetylate the coactivator (Fig. 7*E*, compare *lane 1* with *lane 3*). These acetylation events, however, appear to be noninhibitory for PGC-1α cotranscriptional activity because catalase expression is not reduced from basal levels (Fig. 7*F*, compare *lane 1* with *lane 3*). Taken together, our data provide additional evidence of an important functional relationship between PGC-1α serine 570 and GCN5.

PGC-1α Functions as a Negative Regulator of ROS Elevation and Vascular Hypertrophy in VSMCs—That serine 570 phosphorylation enables PGC-1α acetylation led us to explore the role of PGC-1α post-translational modifications in Ang II-induced, ROS-dependent hypertrophy in VSMCs. Overexpression of the PGC-1α (S570A) mutant completely blocked Ang II-induced increase in H_2O_2 levels, as measured by DCFDA fluorescence (Fig. 8*A*), as well as hypertrophy, as reflected by [^{3}H]leucine incorporation at 24 h (Fig. 8*B*). These findings complement those in Fig. 6*C*, which showed that the PGC-1α (S570A) mutant increased basal catalase expression and prevented Ang II-induced catalase down-regulation. Additionally, we confirmed that the effects of the PGC-1α (S570A) mutant were partially, but significantly, rescued by knockdown of catalase using siRNA (supplemental Fig. S5). Thus, the Akt phosphorylation site-defective PGC-1α mutant blocks Ang II-stimulated increases in ROS levels and hypertrophy in part by preventing Ang II-induced catalase down-regulation. The general inhibitory effects of the PGC-1α mutant overexpression on basal ROS levels and hypertrophy are likely due at least in part to activation of basal catalase expression.

To inform further the inhibitory function of endogenous PGC-1α on ROS production and hypertrophy, we assessed the effects of knockdown of PGC-1α using siRNA. PGC-1α siRNA decreased endogenous catalase protein expression in a dose-dependent manner, suggesting that endogenous PGC-1α upregulates catalase expression basally (Fig. 8*C*), which is consistent with the increase in catalase expression by PGC-1α overexpression (Fig. 3*D*). Both basal and Ang II-stimulated increases in ROS levels and [^{3}H]leucine incorporation at 24 h were enhanced by PGC-1α siRNA as compared with scrambled siRNA (Fig. 8, *D* and *E*). Strikingly, Ang II-induced effects in both scrambled and PGC-1α siRNA-treated VSMCs were abolished by the presence of PEG-catalase, which provides sustained intracellular delivery of catalase to the cells (49). Thus, PGC-1α depletion increases both basal and Ang II-induced

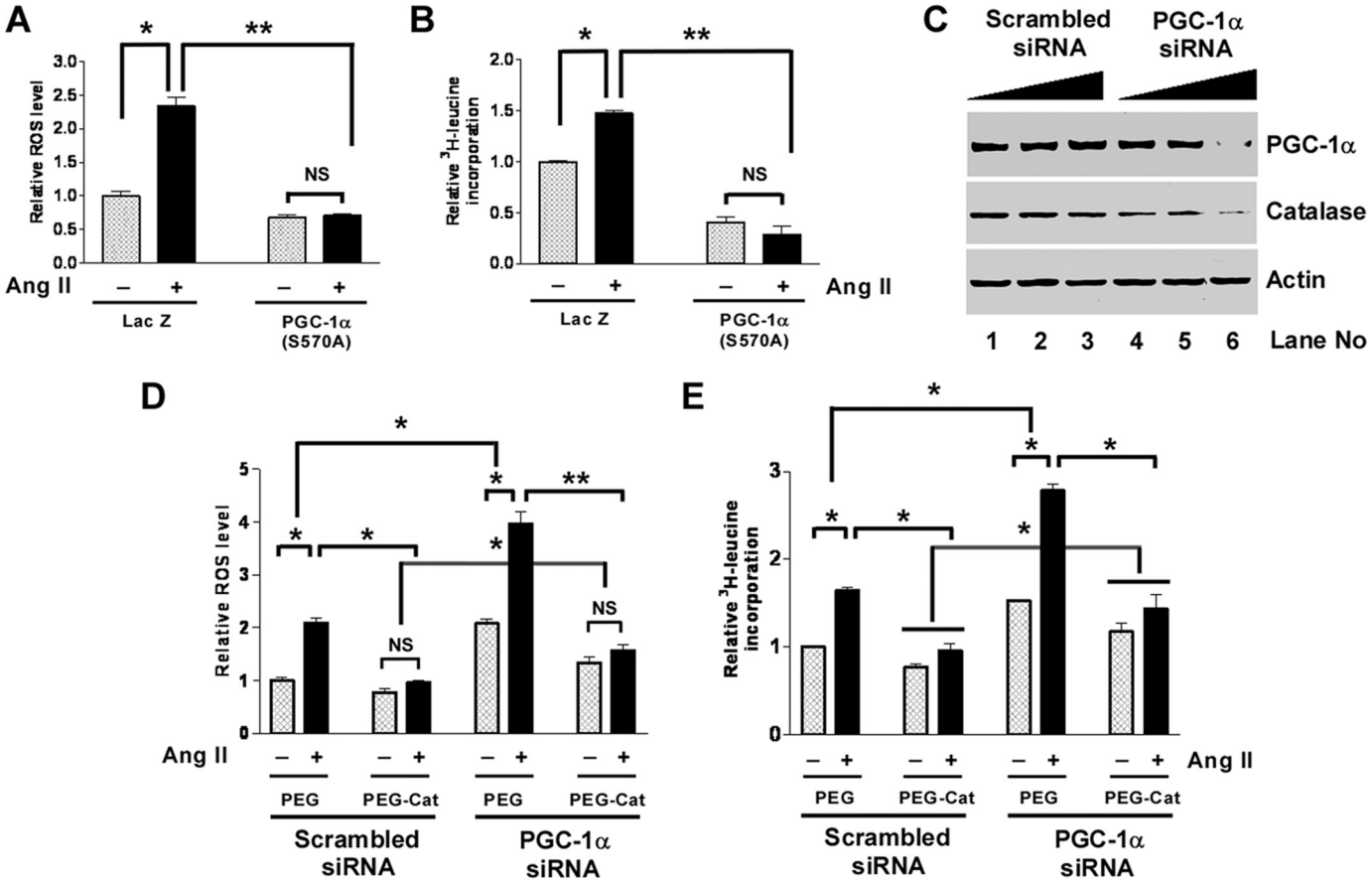

FIGURE 8. **PGC-1α inhibits Ang II-induced increase in ROS levels and vascular hypertrophy.** *A* and *B*, VSMCs infected with Ad.PGC-1α (S570A) or Ad.Lac Z (control) were stimulated with (+) or without (−) 100 nM Ang II for 24 h. Intracellular H_2O_2 levels (*A*) and [^{3}H]leucine incorporation (*B*) were measured as described under "Experimental Procedures." *C*, VSMCs were transfected with increasing concentrations of PGC-1α siRNA or control siRNA (10, 30, or 50 nM) for 48 h. The expression of PGC-1α and catalase was shown by Western blot. *D* and *E*, VSMCs were transfected with 50 nM PGC-1α siRNA or control scrambled siRNA for 48 h. PEG-catalase (100 μnits/ml) or PEG (control, 0.32 mg/ml) was added into the medium for 12 h prior to Ang II stimulation for 24 h. PEG-catalase-inhibitable intracellular ROS levels (*D*) and [^{3}H]leucine incorporation (*E*) were measured as described under "Experimental Procedures." The bar graphs represent averaged data performed in duplicate (means ± S.E.), expressed as fold change over basal ($n = 3–4$). *, $p < 0.05$; **, $p < 0.01$ between the indicated conditions; *ns*, no significant difference.

increases in ROS levels and vascular hypertrophy to an important extent through down-regulating catalase expression.

DISCUSSION

Recent evidence suggests that PGC-1α transcriptional coactivator functions as a master regulator of ROS metabolism; however, its role in vascular hypertrophy and particularly Ang II-induced ROS-dependent vascular hypertrophy has not been investigated. Here we show that Ang II induces Akt-mediated serine phosphorylation and subsequent GCN5-dependent lysine acetylation of PGC-1α, which inhibits PGC-1α transcriptional activity and decreases PGC-1α·FoxO1 complex binding to the FoxO1 response element of catalase promoter, resulting in down-regulation of catalase expression in VSMCs (Fig. 9). FoxO1 is required for PGC-1α-activated catalase expression. Overexpression of the Akt phosphorylation site-defective mutant PGC-1α (S570A) functionally up-regulates catalase expression and inhibits Ang II-induced vascular hypertrophy, at least in part, by reduction of H_2O_2 levels. Ang II stimulation increases ROS levels and vascular hypertrophy, at least in part, by preventing PGC-1α-activated catalase expression resulting from the Akt-mediated serine 570 phosphorylation and subsequent lysine acetylation of PGC-1α by GCN5.

Previous studies demonstrated that PGC-1α is phosphorylated in response to insulin and cytokines in cultured cells (34, 50). Upstream kinases including p38 MAPK (50), AMP-dependent protein kinase (35), and Akt (34) are involved in PGC-1α phosphorylation. The present study shows that inhibitors of p38 MAPK, PI3K, and Akt, but not ERK1/2, as well as overexpression of DN-Akt, significantly inhibited Ang II-induced serine phosphorylation of PGC-1α. Previously, we reported that both PI3K/Akt and p38 MAPK, but not ERK1/2, are activated by Ang II-induced increases in H_2O_2 levels (8, 40) and that Ang II-induced AktP–Akt activation is conferred through p38 MAPK via formation of a redox-sensitive p38 MAPK·MAPK-activated protein kinase 2·Akt complex (46). Our results are in agreement with previous reports indicating that p38 MAPK is an upstream regulator of Akt activation in VSMCs (43–47). Thus, the redox-sensitive p38 MAPK-Akt pathway may play an important role in serine phosphorylation of PGC-1α by Ang II in VSMCs.

Post-translational modifications including phosphorylation and lysine acetylation are involved in the regulation of PGC-1α function and activities in diverse cell types. Little is known about the functional consequences of the interconnections

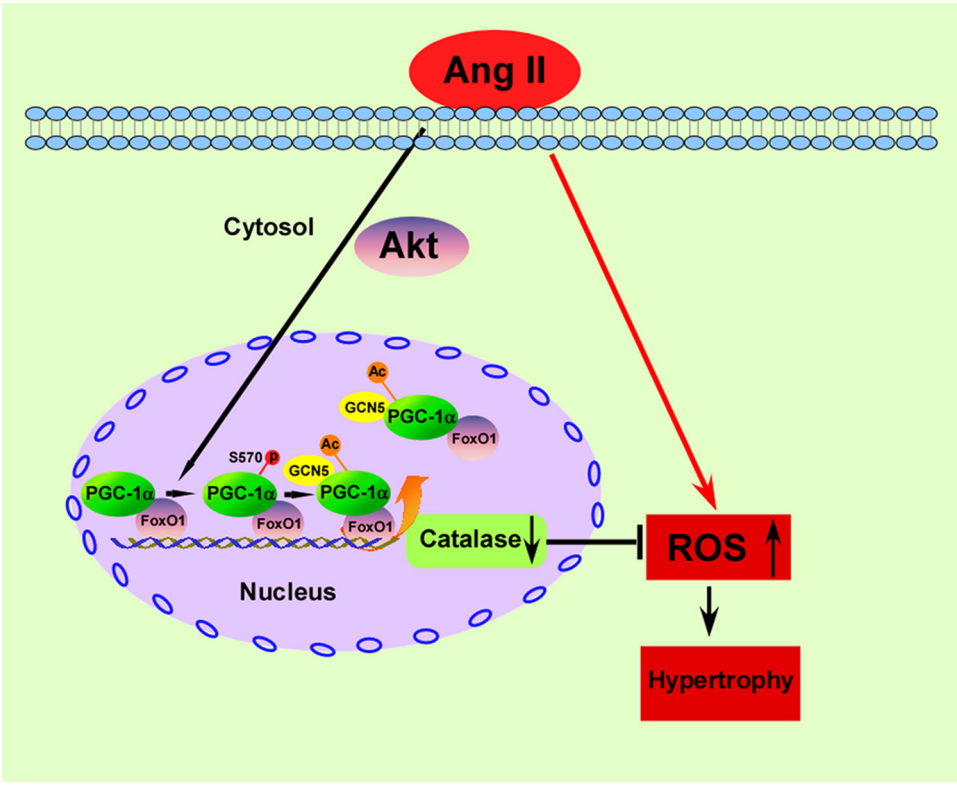

FIGURE 9. **A schematic model for the action and role of PGC-1α in Ang II-mediated ROS-dependent vascular hypertrophy.** Ang II suppresses PGC-1α cotranscriptional activity through Akt-dependent PGC-1α serine 570 phosphorylation, which induces PGC-1α association with GCN5 and results in subsequent lysine acetylation of PGC-1α. These sequential events may promote PGC-1α·FoxO1 complex dissociation from the catalase promoter, which leads to catalase down-regulation. Reduced expression of catalase contributes, at least in part, to long term increase in ROS levels and subsequent vascular hypertrophy.

among those post-translational modifications. Our data provide insights into these inter-relationships. Inhibition of PGC-1α serine phosphorylation by overexpression of the PGC-1α (S570A) mutant prevents both the association of GCN5 with and acetylation of PGC-1α. Thus, the serine 570 phosphorylation site appears to be both necessary and sufficient to regulate PGC-1α acetylation by GCN5. Conversely, a recent report (51) shows that AMP-dependent protein kinase-stimulated phosphorylation of PGC-1α at Ser^{538} and Thr^{177} promotes its activity by facilitating SIRT1-mediated PGC-1α deacetylation. Thus, kinase-specific phosphorylation sites can either promote or inhibit PGC-1α activity by mechanisms involving its acetylation. Our data show that GCN5 depletion prevents Ang II-induced PGC-1α lysine acetylation but not serine phosphorylation and blocks decreases in catalase expression. Thus, lysine acetylation of PGC-1α via GCN5 recruitment appears to be a key post-translational event controlling PGC-1α function and activity by Ang II in VSMC hypertrophy.

The mechanism by which serine 570 phosphorylation promotes acetylation is unknown. Temporally, serine 570 phosphorylation precedes acetylation and appears to be required for Ang II-induced dissociation of PGC-1α·FoxO1 complex from the catalase promoter. Bound, nonphosphorylated serine 570 PGC-1α may be in a conformation in which the N-terminal transcriptional activation domain (amino acids 1–200) to which GCN5 binds is not exposed. Ang II-induced serine 570 phosphorylation may induce a conformational change to expose the activation domain to allow GCN5 binding and to permit acetylation.

Interestingly, knockdown of GCN5 enhances basal acetylation of PGC-1α, suggesting that other acetyltransferases are capable of modifying the coactivator (Fig. 7*E*). These acetylation events, however, appear to have no inhibitory effect on catalase expression because catalase was not down-regulated by Ang II in the absence of GCN5 (Fig. 7*F*). Ang II-induced PGC-1α serine phosphorylation is intact in GCN5-depleted cells. As inferred previously, Ang II-induced acetylation of PGC-1α by GCN5 could be antagonized by SIRT1. In VSMCs, SIRT1 overexpression is able to reverse Ang II-induced PGC-1α sustained acetylation (data not shown). Studies to clarify how SIRT1 and GCN5 coordinately regulate PGC-1α acetylation and identification of PGC-1α potential acetylation sites are under investigation.

Catalase plays an important role in protecting cells from oxidative stress by catalyzing the dismutation of H_2O_2 to oxygen and H_2O. We and others demonstrated that Ang II-induced vascular hypertrophy is mediated by intracellularly produced H_2O_2 and can be blocked by catalase overexpression *in vitro* and *in vivo* (8, 10, 40, 52). Although the functional significance of catalase is evident, relatively little has been known regarding its transcriptional regulation and expression in mammalian cells. In the present study, the ChIP assay reveals that the PGC-1α·FoxO1 complex associates with the catalase promoter regions containing FoxO1-DBE basally *in vivo*, which is prevented by Ang II stimulation. As a consequence, a time-dependent decrease in endogenous catalase expression at both messenger RNA and protein levels is observed. Ang II treatment has no effect on catalase mRNA half-life, thus ruling out a possible effect of Ang II on catalase mRNA stability. These responses induced by Ang II are prevented by p38 MAPK and Akt inhibitors as well as by overexpression of DN-Akt and the PGC-1α (S570A) mutant. Thus, our data are consistent with a model in which Ang II inactivates PGC-1α through Akt-mediated phosphorylation at serine 570 and by subsequent lysine acetylation, thus inhibiting its activity and catalase expression mediated by FoxO1 (Fig. 9). However, the precise mechanism of how post-translational modifications of PGC-1α lead to the dissociation of PGC-1α·FoxO1 complex from the catalase promoter remains to be determined.

Regulation of catalase expression by PGC-1α phosphorylation and acetylation in response to Ang II has functional consequences. Overexpression of Akt phosphorylation site-defec-

tive mutant PGC-1α (S570A) up-regulates catalase expression and inhibits Ang II-induced ROS elevation and vascular hypertrophy, illustrating that the serine 570 site does influence cotranscriptional capabilities directly. We also show that PGC-1α siRNA enhances both basal and Ang II-induced increases in ROS levels and [^{3}H]leucine incorporation, which are reversed by PEG-catalase. These data are consistent with the notion that endogenous PGC-1α functions as a negative regulator of vascular hypertrophy via up-regulation of catalase expression and consequent reduction of ROS levels. In line with our results, Murtaza *et al.* (14) reported that insulin and IGF-1 stimulate cardiac hypertrophy through an increase of ROS by FoxO3a phosphorylation-dependent down-regulation of catalase. These findings are consistent with the concept that a decrease of ROS defense systems can contribute to the elevation of ROS levels in hypertrophic pathways. Thus, Ang II may increase ROS levels and hypertrophy through down-regulation of multiple components of the ROS defense system. Coordinated regulation of PGC-1α Ser570 phosphorylation and lysine acetylation may be a major mechanism by which Ang II induces vascular hypertrophy.

In summary, we demonstrate that Ang II induces Akt-mediated serine 570 phosphorylation and subsequent lysine acetylation of PGC-1α, thereby inhibiting PGC-1α·FoxO1 complex binding to the catalase promoter, which in turn down-regulates catalase expression. Overexpression of PGC-1α (S570A) mutant inhibits Ang II-induced ROS production and vascular hypertrophy via FoxO1-mediated transcriptional up-regulation of catalase. These results suggest that inhibition of the anti-hypertrophic PGC-1α-catalase axis via Akt-mediated serine 570 phosphorylation and lysine acetylation of PGC-1α is an important novel mechanism whereby Ang II increases ROS levels and vascular hypertrophy in VSMCs. These findings provide insight into the possibility that modulating PGC-1α phosphorylation and acetylation may be a viable therapeutic strategy in the broad spectrum of Ang II-mediated, oxidant stress-dependent cardiovascular and metabolic diseases.

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